REMARKS

Brief Description Of The Invention. Human relaxin is a naturally occurring hormone that is produced in the human body in extremely small quantities. Studies using relaxin extracted from animals such as pigs show that the hormone, among other things, plays a role in facilitating birth in mammals, and potentially humans, by relaxing and softening the cervix and reshaping the birth canal. Although crude relaxin samples were first extracted from sow ovaries in the 1930's, prior to Applicants' invention, only insignificant amounts of clinically useful human relaxin have been available. Such human relaxin was primarily obtained from the corpus luteum during pregnancy.

Applicants' invention relates to non-naturally occurring forms of prorelaxin and to a process for producing such prorelaxin. The invention is based upon the unexpected and experimental finding that biologically active relaxin in commercially effective amounts and purity can be produced via non-naturally occurring prorelaxin forms.

By way of background, relaxin is comprised of two interconnected protein chains ("A-chain" and "B-chain"). Relaxin is synthesized in nature as a single chain "preprorelaxin" comprised of a signal peptide, the B-chain, a connecting C-chain and the A-chain. During biosynthesis, the signal peptide is removed, forming "prorelaxin." Further processing occurs *in vivo* in which disulfide bonds are formed between the A- and B-chains and the connecting 110 amino acid C-chain is removed. Applicants discovered unexpectedly that recombinant prorelaxin is processed to a biologically active relaxin in recombinant systems in greater yields than in naturally occurring systems.

Applicants' invention provides the first feasible means for obtaining clinically sufficient amounts of this significant human protein.

Newly Added Claim 33 Is Supported By The Specification. The scope of invention prescribed by newly added Claim 33 is supported in the specification. *See e.g.*, Specification at 2, 28.

Response To The Examiner's Informality Objections. Applicants direct the Examiner's attention to amendments made to the specification and Claim 22 in response to the objections set forth at paragraphs 2 and 4 of the Examiner's June 7, 1994 Office Action (Paper 13).

Response To Objection Of Claims 16-18 and 25-28 Under 37 C.F.R. § 1.821(d). Applicants respectfully traverse the Examiner's objection (Paper 13 at ¶ 3) to Claims 16-18 and 25-28 on the ground that the claims, as amended by the Applicants' July 14, 1993 Preliminary Amendment (*please see*, pages 3-4), recite sequence identifiers in the form of sequence identification numbers ("SEQ ID NOS").

Response To Rejections Under 35 U.S.C. § 112 (first paragraph). The Examiner rejected Claims 1-32 of the instant application for lack of enablement on the grounds that the instant application requires undue experimentation to identify the full range of operative embodiments of the invention as it allegedly does not: (1) provide sufficient guidance as to the length of C-chain or conditions necessary to allow the proper refolding of prorelaxin; and (2) support the use of carboxypeptidase B to excise a C-peptide from a prorelaxin protein. *See*, Paper 13 at ¶¶ 5-6.

Contrary to the Examiner's assertions, one of ordinary skill in the art can readily and easily determine the full range of operative forms of the claimed invention. The standard set forth under § 112 (first paragraph) for determining enablement is one of undue experimentation. *In re Vaeck*, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991). "That some experimentation may be required is not fatal; the issue is whether the amount of

experimentation required is undue." *Id.* Applicants' request reconsideration of the Examiner's rejection for the following reasons:

1. The Examiner's enablement rejection is apparently based upon conjecture that the term "non-naturally occurring C chain," as it appears in the pending claims, is unlimited and may include C-chain's having lengths of one amino acid to "hundreds or thousands of residues." Such unsupported conjecture cannot be the basis of an enablement rejection. In fact, C-chains as short as a few amino acids and as long as 110 amino acids (the length of the naturally-occurring relaxin C-chain) will permit proper refolding of the prorelaxin molecule. The Applicants' choice of the phrase "non-naturally occurring C-chain" was thus purposeful and affords the Applicants the full range of protection to which they are rightly entitled. Applicants offer to submit a declaration regarding the variety of operable C-chain lengths if requested by the Examiner.

Moreover, one of ordinary skill in the art may readily determine whether a prorelaxin having a particular C-chain length is "operative" by routine experimentation. For example, prorelaxin may be screened by any number of means, including chromatography, to determine whether the prorelaxin was "able to properly fold and form the A-chain-B-chain disulfide linkages characteristic of mature relaxins." *See*, Office Action at 2. If the Examiner's reference to the term "operative" to mean biological activity, the Applicants also have provided methods for determining whether a particular embodiment of the invention is biologically active.[1] As more fully set forth at pages 9-10 and 12 (lns. 4-14) of the instant application, a variety of bioassays used to detect relaxin activity have been well-known for at least twenty years. For example, one of the

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[1] Applicants disagree that "operative embodiment" may be equated with biological activity in this instance. The pending claims do not include such limitation. Notwithstanding this disagreement, in an effort to expedite the prosecution of the instant application, Applicants have discussed such contingency.

references cited in the specification disclosing a straight-forward relaxin assay was published in 1960 (Steinetz *et al.*, *Endocrinology* 67:102 (1960)). One of ordinary skill in the art could use any of these known and readily available bioassays to identify whether a prorelaxin molecule having a particular C-chain length is biologically active. Clearly, application of well-known and easily conducted assays constitutes minimal, routine, and not undue, experimentation.

It is respectfully submitted that Applicants' claims are enabled and do not require undue experimentation to identify "operative embodiments."

2. Applicants have identified a range of folding parameters which may be used in the claimed invention. *See e.g.*, Specification at 19. Specifically, the Applicants, by their disclosure, have taught one of ordinary skill in the art that a single chain prorelaxin molecule may be properly refolded under an oxidative environment using a redox buffer.

Prior to this disclosure, one of ordinary skill in the art could not reasonably predict that prorelaxin would properly refold under any conditions, let alone the redox conditions described in the specification. After the Applicants' disclosure, however, one of ordinary skill in the art could reasonably predict that proper refolding of the prorelaxin molecule would occur under oxidative conditions. One of ordinary skill in the art could then refer to any number of redox/protein folding-related publications, to identify any number of conditions under which proper refolding of the prorelaxin molecule would occur.

That Applicants provide only one example and thus one protocol of a folding step does not negate the information disclosed throughout the specification or limit the claimed invention to that one exemplified protocol.

Applicants respectfully request that the Examiner reconsider his rejection in view of the breadth of information set forth in the Applicants' specification.

3. Contrary to the Examiner's assertion that "[t]he specification does not support the use of carboxypeptidase B to excise a C peptide from a prorelaxin protein" on the ground that carboxypeptidase B preferentially cleave arginine and lysine at the carboxy terminus of a polypeptide, Example 5 provides the successful enzymatic cleavage of a mini-C chain from prorelaxin using trypsin in combination with carboxypeptidase B. Specification at col. 32, lns. 21-28, *see also*, Figures 2 and 2A. Applicants request that Examiner withdraw his rejection that the claims are not enabled with respect to the use of carboxypeptidase B.

Response To Rejections Under 35 U.S.C. § 112 (second paragraph).

With respect to the Examiner's comments regarding Claim 1, and corresponding dependent Claims 2-8 and 13-22, Applicants have amended Claim 1 to recite a "refolding step."

With respect to Claim 5, the Applicants assert that the claims language is unambiguous and accurately reflects the cleavage step discussed in the specification, *see e.g.*, page 6, lines 19-29 and at Figure 2A. Applicants nonetheless have amended Claim 5 to further clarify the identification of the enzymes encompassed within Claim 5.

With respect to Claims 16-20 and 25-29, Applicants have amended each claim to be consistent with the Examiner's suggestions.

With respect to the Examiner's separate comments regarding Claims 21 and 22, Applicants have amended each claim to reflect the changes suggested by the Examiner.

The Examiner did not reject Claims 23-24 and 30-32 under 35 U.S.C. § 112 (second paragraph).

Response To Rejections Under 35 U.S.C. § 103. The standard by which obviousness is determined is: (1) whether the invention was suggested in the art; and (2) whether there was a reasonable expectation of success that the invention would work. *In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). The references cited in the June 7, 1994 Office Action each address only the first prong of the obviousness standard set forth by the Federal Circuit and therefore cannot be properly raised as prior art under § 103 against the claims of the present application.

Response To Rejections Of Claims 1-8, 21-24 and 30-32 As Unpatentable Over Hudson et al., In View Of Chang et al. and Enzyme Nomenclature. Hudson, *et al.*, U.S. Patent No. 5,179,195 ("Hudson") discloses a method for making relaxin by chemical synthesis techniques in which the A- and B-chains are separately synthesized and then combined. Hudson therefore does not exemplify a process for making relaxin using a single chain polypeptide, wherein a single polypeptide chain is manufactured and then processed to make prorelaxin and relaxin. As acknowledged by the Examiner, Hudson further does not teach:

(1) "a particular embodiment of a pro- or preprorelaxin having a modified C-chain, DNA encoding it, or its expression in *E. coli*;

(2) "the enzymes which would be advantageously employed to process such a prorelaxin"; or

(3) "cyclization of the N-terminal Gln of the A chain of the mature product."

Rather, Hudson only suggests to one of ordinary skill in the art that the disclosed amino acid and DNA sequences "may" be useful in the recombinant expression of relaxin and/or prorelaxin. Hudson therefore does not provide one or ordinary skill in the art any

reasonable basis for expecting that a modified C-chain would yield an operative prorelaxin or relaxin protein.

Likewise, Hudson, by the chemical synthesis yields reported, does not provide any assurance that use of the disclosed DNA and amino acid sequences would reasonably result in the efficient production of a viable product. According to Hudson, "yields from 1.5 to 6.0% have been achieved as measured by biological activity" in animal assays using this process. *See also*, cols. 16-17 wherein 5.3% and 3.0% yields were also reported. Hudson is further silent as to whether alterations to the cleavage sites would affect the folding pattern of the A- and B-chains of prorelaxin and thus the mature product's biological activity. Hudson simply does not provide one skilled in the art a reasonable expectation of success that the recombinant expression of a polypeptide from a non-naturally-occurring DNA sequence would result in a biologically active relaxin protein.

Neither Chang *et al., BBRC 171*:818-826 (1990) ("Chang") nor *Enzyme Nomenclature* (1984) remedy this deficiency. Both Chang and *Enzyme Nomenclature* identify enzymes and their known cleavage patterns. Thus, neither provide any insight as to whether a protein having altered cleavage sites or an altered C-chain would be active or whether a single-chain process is viable in a recombinant process.

The Examiner's hindsight reconstruction of the present claims with references that provide only the impetus "to try" and not reasonable assurance of success, to support his obviousness rejection is impermissible and respectfully traversed.

Response To Rejections Of Claims 14 and 15 As Unpatentable Over Hudson et al., In View Of Chang et al., Enzyme Nomenclature, Stults et al. and Dimarchi et al. Claims 14 and 15 depend on independent Claim 1. Stults *et al.* does not

remedy the short-comings of Hudson, Chang and the *Enzyme Nomenclature* references. To the extent that Claim 1 is not obvious as the success of the claimed process was not reasonably expected, Claims 14 and 15, which claim the same process but address the cyclization of the A-chain's N-terminal Gln are also not obvious.

Response To Rejections Of Claims 1-8, 21-24 and 30-32 As Unpatentable Over Hudson et al., In View Of Chang et al., Enzyme Nomenclature and Olson. For the reasons set forth above, Olson, which teaches techniques for obtaining heterologously expressed proteins, does not render obvious the claimed invention.

Applicants acknowledge and concur with the Examiner's finding that "Claims 16-20 and 25-29 are free of the prior art."

An early and favorable action is requested.

Respectfully submitted,

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